Feeding by Waterhyacinth Weevils (*Neochetina* spp.) (Coleoptera: Curculionidae) in Relation to Site, Plant Biomass, and Biochemical Factors

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ABSTRACT Biological control of waterhyacinth [*Eichhornia crassipes* (Mart.) Solms.] by waterhyacinth weevils [*Neochetina bruchi* (Hustache)] and [*Neochetina eichhorniae* (Warner)] varies according to field environment, season, and plant stress levels. Plants at four field sites were sampled to determine if leaf scarring caused by adult weevil feeding was associated with plant biomass, biochemical and population measures, and insect densities. Leaf scar densities were consistently higher on plants from two sites at which root and dead plant part biomass were high. Scarring was correlated to root and dead biomass across all sites. Scarring was not associated with weevil densities summed across all life stages or larval gallery density. Soluble protein contents were lower in plants from the two sites with high scarring than at two other sites in Spring 2002, and scarring was negatively correlated to protein content across all sites. Protein was usually highest in immature furled leaves. Activities of soluble peroxidase enzymes were highest in old leaves. Scar densities were not associated with canopy height and shoot density. At one site, high scar densities occurred on plants with small leaf areas, which were likely growing slowly under the influence of multiple abiotic and biotic stress factors. Mechanical and natural plant removal and regrowth may have facilitated plant compensation for weevil feeding at the other site with high scarring. Temporally and spatially dynamic physical and biochemical plant traits and growth environments could limit biological control of waterhyacinth.

KEY WORDS Pontederiaceae, aquatic weed, plant quality, damage

INSECT HERBIVORY ON PLANTS occurs in the context of variation in host plant quality, which can influence both the perception of a plant as a host (Bernays and Chapman 1994), and the ability of insects to develop and reproduce (White 1984, Karban and Baldwin 1997, Awmack and Leather 2002). Stressful conditions may limit plant growth and defense production and alter nutrient intake by herbivores (Herms and Mattson 1992, Price 2000). Low nutrient availability (Herms and Mattson 1992) and pathogen infection (Hatcher 1995) are examples of environmental factors that can influence herbivory, leading to heterogeneity in herbivore abundance and damage on plants.

Biological control of exotic weeds with introduced herbivores involves insect–plant associations in which predation is reduced or absent, potentially increasing the importance of variation in host weed quality (Price 2000). Agents may vary in abundance and impact in the field. Examples include two tephritid flies on diffuse knapweed (*Centaurea diffusa* L.) (Myers and Risley 2000), a specialist noctuid caterpillar on waterlettuce (*Pistia stratiotes* L.) (Wheeler et al. 1998), and weevils and moths on giant salvinia (*Salvinia molesta* Mitchell) (Room et al. 1989). Defensive or adaptive responses by plants to both the environment and to agent feeding may be responsible (Zidack 1999). Variable leaf nitrogen content and toughness were important to larval survival in waterlettuce (Wheeler et al. 1998). High nitrogen and vigorous plant growth increased biocontrol effects in giant salvinia (Room et al. 1989). Knowledge of such plant-based variation is clearly important in evaluating releases and predicting effects on plants.

Biological control of waterhyacinth [*Eichhornia crassipes* (Mart.) Solms.], a floating aquatic weed, involves two imported waterhyacinth weevil species (*Neochetina bruchi* Hustache and *Neochetina eichhorniae* Warner) (Coleoptera: Curculionidae) (DeLoach and Cordo 1976, Center et al. 1999b) among other agents. These weevils have eliminated the need for widespread use of other control methods at locations in the Unites States (Goyer and Stark 1984, Haag and Center 1988), Australia, India, Africa (Wright 1980, El Abjar and Beshir 1984, Jayanth 1988), and the plant's native range in Argentina (DeLoach and Cordo 1983). Economically and/or ecologically damaging plant populations still occur in the United States (Center

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and Dray 1992, Grodowitz et al. 2000). Waterhyacinth weevil abundance varies among field sites (Center et al. 1999a, Grodowitz et al. 2000). Possible causes include chemical or mechanical removal (Center and Durden 1986) and changes in plant growth stage, which influence weevil sex ratio and flight muscle development (Center and Dray 1992, Center et al. 1999a). Adult weevils feed mostly on furled and partially unfurled young leaves emerging from the central rhizome, in part because of the high nitrogen content of these leaves (Center and Wright 1991). Weevil feeding itself alters nutrient profiles and leaf toughness (Center and Van 1989, Wright et al. 1989). Oxidative enzymes such as peroxidases contribute to lignification processes in crop plants, which lead to toughening of plant tissues (Nicholson and Hammerschmidt 1992) and in some cases altered resistance to herbivores (Bi and Felton 1995).

In the lower Rio Grande Valley of Texas, biological control of waterhyacinth by waterhyacinth weevils varies greatly (Grodowitz et al. 2000), and field populations differ in water flow and use and in weed management practices. The objective of this study was to determine whether leaf scarring by adult weevils on leaves was associated with leaf area, plant size, biomass and population measures, weevil densities, and two physiological indicators of plant quality, protein content and peroxidase activity.

Materials and Methods

Description of Field Sites. Four field sites in the lower Rio Grande Valley of Texas were chosen to encompass variation in water use and the presence of mechanical weed control. All sites were located within ≈60 km of each other (latitude 26°03–24 min N, 97°34–57 min W). Water depths at sampling points ranged from 0.3 to 3.0 m. Site LC (Lake Canal) was a continuously flowing canal connecting two sides of a reservoir. Plant movement occurred at this site because of the water current. No mechanical removal occurred. Site RG (Rio Grande) was located at the mouth of a pumping station inlet on the river. Plants were mechanically removed at this site by irrigation district personnel early in the study. Natural plant movement occurred because of variable water flow in the river. Site IN (Inlet) was an abandoned water pumping inlet adjacent, but not connected, to the Rio Grande. Water flow was minimal, and no mechanical control occurred. Site BP (Bypass Canal) was an irrigation canal adjacent to a small reservoir. Mechanical removal occurred at this site in September 2001. Plant replacement occurred throughout the study during occasional water releases.

Field Site Sampling. All four sites were sampled during Summer 2001 (27 June-17 July 2001), Fall 2001 (6–13 November 2001), Spring 2002 (4–9 April 2002), and Summer 2002 (11–15 July 2002). Water temperatures ranged from 24.6 (Fall 2001) to 30.6°C (Summer 2002). Plastic pipe squares (0.5 m by 0.5 m) were thrown into mats to define 0.25-m^2 sampling units (five sampling units per field site in Summer 2001 and four per site at other times). In each unit, canopy height (to the apical tip of the tallest leaf) and shoot ramet density (Center et al. 1999a) per meter squared were determined. A waterhyacinth shoot was defined as a rosette with root initials, whether free-floating or a stoloniferous daughter plant. Ten to 15 shoots per sampling unit (depending on availability) were selected without apparent bias and individually bagged for dissection. Three additional plants per unit were provided with enough water (taken from the site) to cover the roots and were sampled for protein and peroxidase measures.

Plant Assessment. Bagged plants were washed, blotted dry, and divided into biomass components. Above-water biomass consisted of all live leaf laminae and petioles and the central rhizome of the rosette (not including connecting stolons). Dead biomass consisted of leaves that were 50% or more dead (determined by visually estimating the brown portion of total laminar and petiolar area) and the petiolar bases of dehisced leaves. Root biomass consisted of all available root matter. Above-water biomass was divided by root biomass to estimate shoot-to-root ratios. In five randomly selected plants, numbers of leaves were counted and the lengths of the two youngest unfurled leaves (lamina and petiole) measured. Leaf laminar area was determined with a LI-3100 leaf area meter (Li-Cor, Lincoln, NE). In Fall 2001, Spring 2002, and Summer 2002 samples, numbers of beetle feeding scars on the adaxial leaf laminae of the two youngest unfurled leaves were counted and divided by leaf area to determine scar density. At all sampling times, numbers of galleries in the petioles of all leaves were counted and expressed on a per leaf basis. Most galleries were the result of feeding by waterhyacinth weevil larvae, although a few were made by waterhyacinth moths [*Niphograpta albiguttalis* (Warrren) (Lepidoptera: Pyralidae)]. Weevil adults, larvae, and eggs were counted throughout the plant. Because of low adult counts (0–0.2 adults per leaf), egg, larval, and adult weevil densities were summed.

Analysis of Soluble Protein Content and Peroxidase Activity. The furled, youngest unfurled, and oldest unfurled leaves (50% or less senescent brown area on the lamina) were excised from three plants per unit, pooled, frozen in dry ice, and stored at −80°C. Leaves were ground on dry CO_2 ice. Samples (0.2–0.5 g) were homogenized in 0.01 M sodium phosphate buffer (pH = 7; 10 ml/g fresh tissue weight) containing 0.75 mM ethylenediaminetetraacetic acid (EDTA) and 1% (wt:vol) polyvinylpyrrolidone. Extracts were centrifuged at 11,000 rpm for 15 min. Fifty microliters of supernatant was mixed with 1.5 ml Brilliant Blue G reagent (Sigma, St. Louis, MO) and incubated for 5 min at 25°C to measure total soluble protein, using the method of Bradford (1976), at 595 nm in a GeneSys-2 spectrophotometer (Spectronic, Rochester, NY). Bovine serum albumin was used as a standard, and protein content was expressed in milligrams per gram fresh weight. Peroxidase activity was measured in a 1.5-ml reaction mixture (150 μl protein extract and 1,350 μl 0.025 M phosphate buffer, pH = 7, containing

Table 1. Contrasts from repeated measures ANOVA of leaf scar density resulting from *Neochetina* spp. weevil feeding and for other variables potentially associated with scarring

	Factor												
	Site			Time			Site×time			Time×time[a]		Time²×site[a]	
	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*	*F*	*P*	*F*	*P*
Damage on Y1	3, 12	27.5	<0.001	1,24	8.44	0.008	3, 24	0.27	0.848	9.21	0.006	13.0	<0.001
Damage on Y2	3, 12	49.5	<0.001	1,24	10.3	0.005	3, 24	0.95	0.474	5.29	0.010	1.38	0.134
Leaf area-Y1	3, 12	20.9	<0.001	1,24	0.81	0.377	3, 24	3.53	0.030	16.3	0.001	4.35	0.014
Root biomass	3, 16	19.3	<0.001	1,40	23.4	<0.001	3, 40	7.70	<0.001	24.6	<0.001	14.37	0.010
Dead biomass	3, 16	6.42	0.005	1,40	0.12	0.730	3, 40	0.78	0.510	1.30	0.262	1.70	0.182
Above-water biomass	3, 16	7.78	0.002	1,40	0.01	0.923	3, 40	0.91	0.444	21.07	<0.001	7.05	<0.001
Weevil density	3, 16	8.21	0.002	1,37	6.89	0.013	3, 37	3.42	0.027	7.52	0.009	2.70	0.060
Galleries	3, 16	20.9	<0.001	1,40	18.7	<0.001	3, 40	4.61	0.007	2.62	0.114	8.48	<0.001
Protein-F	3, 16	25.6	<0.001	1,40	0.34	0.562	3, 40	2.82	0.051	15.4	<0.001	21.5	<0.001
Protein-Y	3, 16	37.8	<0.001	1,40	11.9	0.001	3, 40	1.88	0.149	61.0	<0.001	21.7	<0.001
Peroxidase-F	3, 16	2.09	0.142	1,36	1.56	0.219	3, 36	0.92	0.441	0.09	0.761	0.40	0.750
Peroxidase-Y	3, 16	4.66	0.016	1,35	5.38	0.026	3, 35	6.26	0.002	19.6	<0.001	2.28	0.096
Canopy height	3, 16	5.71	0.007	1,40	4.16	0.048	3, 40	0.29	0.831	0.13	0.724	0.45	0.565
Shoot density	3, 15	2.25	0.125	1,40	0.48	0.493	3, 40	1.43	0.249	0.50	0.482	1.29	0.290

Damage on Y1 and Y2, damage to laminae by waterhyacinth beetles on the youngest (Y1) and next-to- youngest (Y2) unfurled leaf; weevil density, combined egg, larval and adult insects per leaf; protein/peroxidase-F and -Y, protein content or peroxidase activity in furled (F) and youngest unfurled (Y) leaves.

[a] Degrees of freedom for the time × time and time² × site effects are equal to those for the site and site × time effects, respectively.

0.25% guaiacol [vol:vol] substrate and 0.375% hydrogen peroxide [vol:vol]). The reaction was monitored for 1 min at 470 nm at 25°C, and the linear change in absorbance was determined as a measure of activity per gram fresh weight.

Statistical Analyses. Averages taken from all plants examined in each sampling unit were used in all analyses ($n = 4$–5 units per site per sampling time). All data were examined for normality using PROC UNIVARIATE and Wilks lambda (SAS Institute 1999). Data were $\log(x + 1)$ transformed for analysis to obtain a fit to a normal distribution. Repeated measures analysis (PROC MIXED, SAS Institute 1999) with residual maximum-likelihood estimation was used to examine variation across three sampling times in feeding scar density and leaf area. Similar analyses across four times examined plant biomass, weevil and gallery densities, protein, and peroxidase measures in furled and young leaves, canopy height, and shoot density. Site and sampling time were main effects, and the repeated subject factor was sampling unit nested within site. Simple covariance or unstructured covariance with banding minimized Akaike's finite sample information criterion (AICC; SAS Institute 1999). Type 1 *F*-tests were used to examine significance of site, sampling time, their linear interaction, and nonlinear interaction terms involving sampling time.

Pearson correlations were used to test for associations between laminar scarring on the youngest unfurled leaf and plant size, biomass, and population measures and plant chemical factors (PROC CORR, SAS Institute 1999), both at individual sampling times and across all times. Within each time, differences among sites were assessed with site as a fixed effect in analyses of variance (ANOVAs) and Tukey mean comparisons ($P < 0.05$) using PROC GLM (SAS Institute 1999). The effects of site and leaf age on protein content and peroxidase activity were assessed with two-factor ANOVA.

Results

Leaf Scarring by Beetles. The amount of *Neochetina* spp. weevil feeding damage on the two youngest unfurled leaf laminae of waterhyacinth plants differed significantly among field sites and sampling times (Table 1). Scarring on youngest unfurled leaves was lower at all sites in Spring 2002 samples than in Fall 2001 samples (average 53% decrease; Fig. 1). Scarring increased 1.9-fold over Spring 2002 levels by Summer 2002 on plants from site IN, whereas they decreased or showed little change between these two times at the other three sites. Scar densities on the second-youngest unfurled leaves paralleled densities on youngest leaves (Fig. 1; Table 2; across all three sampling times, $r = 0.91$, $n = 48$, $P < 0.001$). Scarring varied significantly by site at each sampling time (Summer 2002: youngest unfurled leaf, $F = 13.1$, df = 3, 12, $P < 0.001$; second-youngest leaf, $F = 13.2$, df = 3, 12, $P < 0.001$). Across three sampling times, scar densities on plants from sites IN and BP were 3.7- to 8.9-fold greater (youngest unfurled leaves) and 2.9- to 10.7-fold greater (second-youngest leaves) than densities on plants at sites LC and RG. Densities were more consistently elevated at site IN than at site BP (Fig. 1).

Smaller leaf areas could have resulted in greater scar density at sites IN and BP compared to LC and RG. The area of the youngest unfurled leaf varied significantly by site across all four sampling times (Table 1) and at each sampling time (Summer 2002: $F = 14.3$, df = 3, 12, $P < 0.001$). The youngest unfurled leaves at site IN were always smaller (43–81%) than leaves of the same age at LC and RG, with significant differences in Summer 2001 and Spring 2002 samples (Fig.

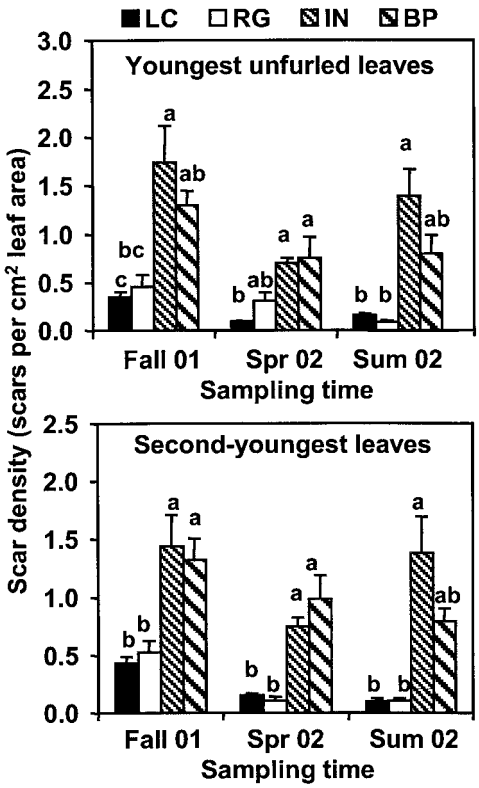

Fig. 1. Leaf scarring damage made by *Neochetina* spp. beetle adults to the laminae of the two youngest unfurled leaves of waterhyacinth plants sampled from four sites at three times (Spr 02, Spring 2002; Sum 02, Summer 2002). Each bar represents the mean ± SE of four samples. Means marked with the same letters within each sampling time are not significantly different at $P < 0.05$.

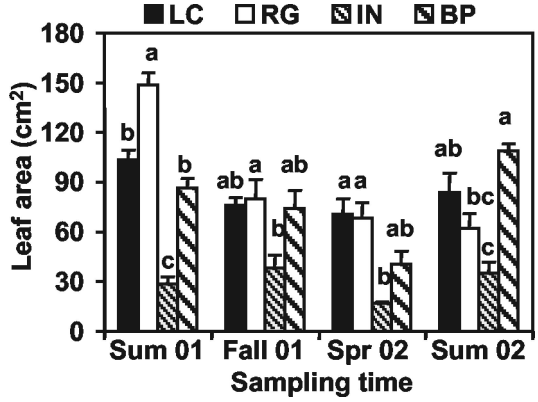

Fig. 2. Area of the lamina of the youngest unfurled leaf of waterhyacinth plants at four field sites sampled at four times (Sum 01, Summer 2001; Spr 02, Spring 2002; Sum 02, Summer 2002). Each bar represents the mean ± SE of four to five samples. Means marked by the same letters within each sampling time are not significantly different at $P < 0.05$.

2). Youngest unfurled leaves at site IN were also usually smaller (49–68%) than leaves of the same age from site BP (Fig. 2). The laminar areas of second-youngest unfurled leaves were strongly correlated to areas of youngest leaves (across all four sampling times, $r = 0.95$, $n = 64$, $P < 0.001$).

Associations Between Leaf Scarring and Plant Biomass. Three components of waterhyacinth biomass (roots, dead plant parts, and total live above-water plant parts) varied significantly between field sites across all sampling times (Table 1). Plants with high amounts of root biomass tended to have high dead biomass (across all sampling times, $r = 0.71$, $n = 64$, $P < 0.001$) and high above-water biomass ($r = 0.55$, $n = 64$, $P < 0.001$). Only root biomass varied significantly over time and showed a site by time interaction (Table 1). Root biomass increased (1.3- to 2.5-fold) in plants at all sites between Spring 2002 and Summer 2002 sampling times (Fig. 3). Patterns involving more than two sampling times likely explain the significant nonlinear (time × time and time^2 × site) factors in Table 1. Both root and above-water biomass were higher in Fall 2001 than in Summer 2001 in plants from the two sites with high leaf scarring (IN and BP), decreasing at these two sites by Spring 2002, and increasing again by Summer 2002 (Fig. 3). This pattern was not apparent in plants from sites LC and RG.

In Fall 2001 and Summer 2002 samples, dead biomass was positively associated with scarring on youngest unfurled leaves across all sites (Table 2; across all three times, $r = 0.60$, $n = 48$, $P < 0.001$; Fig. 4), and root biomass showed a similar association (Table 2; across all three times, $r = 0.49$, $n = 48$, $P < 0.001$). In Summer

Table 2. Correlations occurring at one or more sampling times between damage to the lamina of the youngest unfurled leaf of waterhyacinth plants and plant biomass, damage, and chemical measures

Variable	Sampling time								
	Fall 2001			Spring 2002			Summer 2002		
	r	*P*	*n*	*r*	*P*	*n*	*r*	*P*	*n*
Damage on Y2	0.88[a]	<0.001	16	0.90[a]	0.001	16	0.94[a]	<0.001	16
Root biomass	0.65[a]	0.006	16	0.17	0.538	16	0.44	0.086	16
Dead biomass	0.60[a]	0.010	16	0.28	0.301	16	0.75[a]	0.001	16
Galleries in petioles	−0.57[a]	0.020	16	0.27	0.310	16	0.15	0.593	16
Protein-furled leaves	−0.37	0.158	16	−0.75[a]	0.001	16	−0.50	0.050	16
Protein-young leaves	−0.33	0.211	16	−0.72[a]	0.002	16	−0.43	0.095	16
POD-furled leaves	−0.53[a]	0.034	16	−0.06	0.825	16	−0.16	0.548	16

POD, peroxidase. *n*, sample size.
[a] Significant correlation.

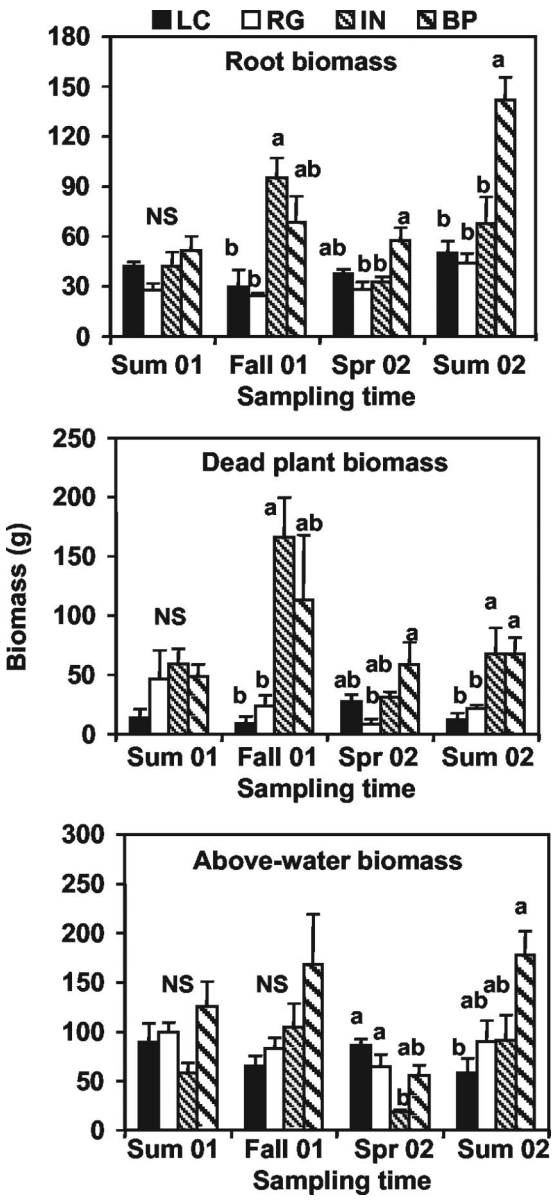

Fig. 3. Biomass of waterhyacinth plants at four field sites. Sampling time abbreviations as in Fig. 2. Each bar represents the mean ± SE of four to five samples. Means marked by the same letters within each sampling time are not significantly different at $P < 0.05$. NS, no significant differences between sites.

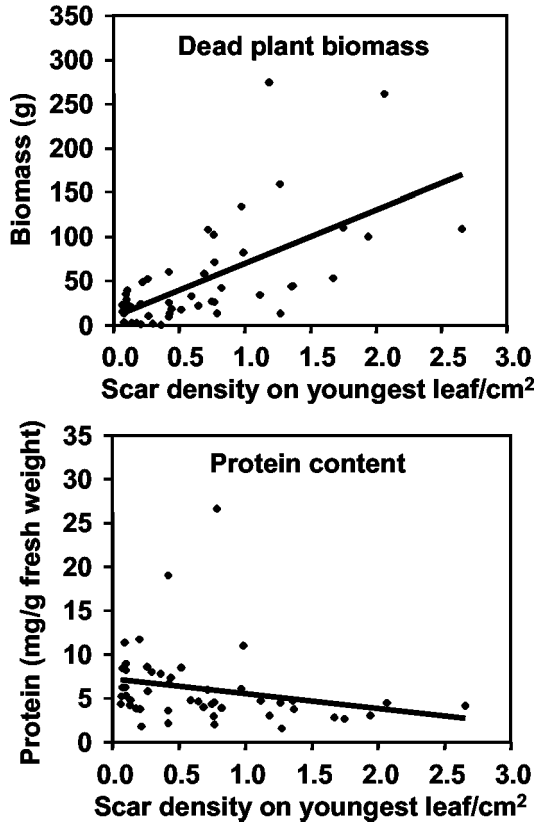

Fig. 4. Plots of leaf scarring by waterhyacinth weevils against biomass-based and biochemical indicators of the impact of weevil feeding. Each data point represents the average from a sample of five plants; $n = 48$ samples from three sampling times (Fall 2001, Spring 2002, and Summer 2002).

2002, root and dead biomass were significantly higher (roots, 3.0-fold; dead, 4.4-fold) in plants from site BP than from the two sites with lower scarring, LC and RG (Fig. 3; roots, $F = 16.1$, df = 3, 12, $P < 0.001$; dead, $F = 4.9$, df = 3, 12, $P = 0.019$). Dead biomass at site IN showed a similar difference at this time (Fig. 3). In Fall 2001, plants from site IN had higher root and dead biomass than plants at sites LC and RG (roots, 3.5-fold; dead, 13-fold; roots, $F = 9.25$, df = 3, 12, $P = 0.002$; dead $F = 5.33$, df = 3, 12, $P = 0.015$). Above-water biomass varied between sites at two of four sampling times (Fig. 3; Summer 2002, $F = 5.57$, df = 3, 12, $P = 0.013$) but was not correlated with scarring ($P > 0.05$) and did not vary between pairs of sites with relatively high (IN, BP) and low (LC, RG) scar densities (Fig. 3). Shoot-to-root ratios in plants at sites IN (0.77 ± 0.08) and BP (1.42 ± 0.26) were lower than at sites LC (2.62 ± 0.25) and RG (2.59 ± 0.20) in Spring 2002 ($F = 18.5$, df = 3, 12, $P < 0.001$). Ratios did not differ among sites at other times.

Associations Between Leaf Scarring and Weevil Densities. Densities of *Neochetina* spp. weevils per leaf (eggs, larvae, and adults combined) and numbers of larval galleries per leaf petiole varied significantly by site across all sampling times and over time (Table 1). However, neither measure was correlated to the amount of scarring on the youngest unfurled leaf (Table 2; across all three times, $P > 0.05$). Weevil (Fig. 5) and gallery density on plants from both sites IN and BP did not differ consistently from densities on plants from the other two sites.

Associations Between Leaf Scarring and Protein and Peroxidase. Protein concentrations in furled and youngest unfurled leaves varied by site across all four

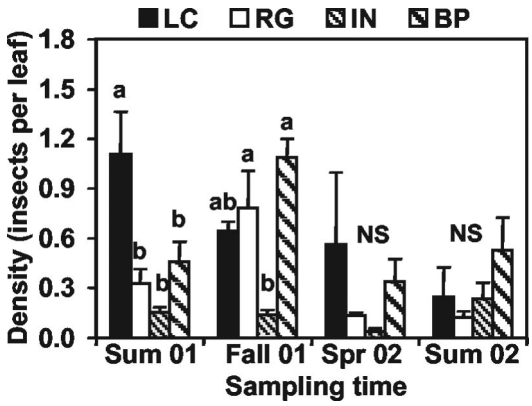

Fig. 5. Density of *Neochetina* spp. weevils on waterhyacinth plants at four sites. Sampling time abbreviations as in Fig. 2. Each bar represents the mean ± SE of four to five samples. Means marked by the same letters within each sampling time are not significantly different at $P < 0.05$. NS, no significant differences between sites.

sampling times (Table 1). Protein content in furled leaves was negatively associated with leaf scarring on youngest unfurled leaves in Spring and Summer 2002 samples (Table 2; across all three times, $r = -0.34$, $n = 48$, $P = 0.02$; Fig. 4). In Spring 2002, plants at sites IN and BP had lower (60%) protein content in furled leaves than plants at sites LC and RG ($F = 48.0$, df = 3,12, $P < 0.001$; Table 3). In a similar nonsignificant trend, sites IN and BP were 57% lower than the other sites in Fall 2001. Protein content in youngest unfurled leaves was negatively associated with scarring in Spring 2002 (Table 2) but not across all times ($P > 0.05$). Plants from site BP had lower (72–79%) protein at this time ($F = 45.0$, df = 3, 12, $P < 0.001$) than did plants from site LC or RG (Table 3). Across all sites and most times, protein contents were significantly higher in furled leaves than in youngest unfurled leaves (1.2- to 1.4-fold difference) and oldest unfurled leaves (1.4- to 1.5-fold difference; Table 3). (Summer 2002: $F = 25.3$, df = 11, $P < 0.001$; leaf age effect, $F = 8.32$, df = 2, $P = 0.001$)

Peroxidase activity in youngest unfurled but not furled leaves varied significantly by field site across all sampling times (Table 1) and also in time and site × time interaction effects. Activities in these leaves were not correlated with leaf scar densities (Table 2; across all three sampling times, $P \geq 0.05$), and plants at sites IN and BP did not differ in activity from plants at the sites with less scarring (Table 4). Peroxidase activities were 13- and 10-fold higher in Fall 2001 samples than in Spring and Summer 2002 samples, respectively, across all sites and leaf ages. Peroxidase was consistently highest in old leaves (Table 4). Activities in these leaves were elevated 15.3-fold over activities in furled leaves and 7.8-fold over youngest unfurled leaves. (Summer 2002: $F = 3.80$, df = 11,34, $P = 0.001$; leaf age effect, $F = 14.3$, df = 2, $P < 0.001$)

Associations Between Leaf Scarring and Plant Population Measures. Waterhyacinth canopy height varied significantly across field sites and over time, whereas shoot density did not vary (Table 1). Plants from populations with high canopy heights tended to have high total above-water biomass (across all times, $r = 0.54$, $n = 64$, $P < 0.001$). Shoot density was negatively correlated to root biomass at two of four sampling times (across all times, $r = 0.33$, $n = 63$, $P = 0.008$) and to above-water biomass at all times ($r = 0.63$, $n = 63$, $P < 0.001$). Canopy height was thus an indicator of large plant size. Shoot density may have reflected levels of asexual plant reproduction and shoot crowding. Neither measure was correlated with the amount of scarring on youngest unfurled leaves at individual sampling times ($P > 0.05$), and neither varied in a consistent, significant manner between site pairs IN and BP and LC and RG (Fig. 6).

Discussion

Variation among sites in adult waterhyacinth weevil damage is a common feature of field survey studies (Center and Durden 1986, Center et al. 1999a). Sites IN and BP shared high scar densities (Fig. 1) but differed in both environmental factors such as mechanical control and in leaf size and plant biomass.

Table 3. Total soluble protein content (mg/g fresh weight, mean ± SE) in furled, youngest unfurled (young), and oldest unfurled (old) leaves of waterhyacinth

Leaf age	Site	Sampling time[a]		
		Fall 2001	Spring 2002	Summer 2002
Furled	LC	10.2 (3.5)	9.22 (0.7)a	4.38 (0.4)
	RG	12.4 (4.8)	7.05 (0.4)b	4.98 (0.5)
	IN	4.76 (0.4)	4.40 (0.2)c	3.70 (0.5)
	BP	5.12 (2.0)	2.05 (0.3)d	4.54 (0.5)
Young	LC	5.51 (1.0)	7.38 (0.5)a	2.94 (0.1)b
	RG	7.99 (1.1)	5.71 (0.2)b	4.67 (0.2)a
	IN	6.55 (2.1)	4.33 (0.5)b	3.04 (0.2)b
	BP	4.57 (0.7)	1.58 (0.3)c	2.73 (0.6)b
Old	LC	7.28 (1.5)	8.36 (0.8)a	2.58 (0.2)b
	RG	7.01 (1.2)	4.62 (0.4)b	5.11 (0.8)a
	IN	6.56 (0.9)	4.60 (0.4)b	3.07 (0.2)ab
	BP	7.75 (1.2)	1.24 (0.4)c	1.96 (0.7)b

Table 4. Peroxidase activity (ΔAbs_{470} per min/g fresh weight, mean ± SE) in furled, youngest unfurled (young), and oldest unfurled (old) leaves of waterhyacinth

Leaf age	Site	Sampling time[a]		
		Fall 2001	Spring 2002	Summer 2002
Furled	LC	57.3 (40)a	6.59 (1.9)	3.53 (0.7)
	RG	25.7 (3.5)ab	12.2 (3.8)	27.1 (21)
	IN	14.6 (4.5)ab	7.15 (0.7)	7.41 (1.1)
	BP	7.61 (2.4)b	9.07 (2.2)	10.6 (1.4)
Young	LC	193 (3.5)	10.6 (0.6)ab	3.61 (1.0)b
	RG	34.5 (6.3)	23.5 (6.9)a	10.1 (1.8)a
	IN	31.3 (5.9)	14.9 (2.3)ab	11.3 (1.5)a
	BP	24.3 (14)	7.70 (1.9)b	8.78 (1.5)a
Old	LC	507 (39)	28.5 (3.8)ab	32.6 (12)
	RG	1618 (1200)	42.5 (5.8)a	53.7 (13)
	IN	274 (141)	52.6 (8.2)a	63.4 (10)
	BP	150 (57)	11.2 (5.2)b	62.9 (57)

Leaf scarring occurred in the context of seasonal and plant phenological influences on waterhyacinth. The time- and leaf age-related trends in total protein content and peroxidase activities (Tables 3 and 4) and provide insights about adult weevil preferences. Correlation analyses suggest that weevil feeding influenced leaf biochemistry and biomass allocation in plants within sites. Scar density was not sufficient to explain variation between sites in plant biomass and population measures.

Small leaf laminar areas (Fig. 2) contributed to the elevated scar densities at site IN by reducing surface areas available for feeding. Waterhyacinth weevil densities were not elevated at site IN relative to other sites and were low at all sites (Fig. 5). High scar densities at site IN could have reflected accumulation of feeding damage in slow-growing plants. However, numbers of leaves per plant did not vary by site (P. J. M., unpublished data). Leaf retention and turnover were likely not different in plants at site IN than in plants at other sites. The chronically small leaf areas suggest the presence of stress from abiotic factors (Gopal and Sharma 1981), weevil feeding (DeLoach and Cordo 1983, Center et al. 1999b), and/or pathogen infection (Charudattan et al. 1978). The high root and dead plant biomass at site IN, in the absence of strong differences between sites in above-water live biomass (Fig. 3), is also suggestive of stress (Gopal and Sharma 1981). Damage accumulation and stress and seasonal effects on plant growth (Grodowitz et al. 1991) could explain why root and dead biomass were especially high at site IN late in the growth season (Fall 2001 samples). Biological control stress in waterhyacinth impacts leaf growth more rapidly than root growth (Charudattan et al. 1978, Grodowitz et al. 1991). Dead biomass often increases after feeding by *Neochetina* spp. weevils (Goyer and Stark 1984, Center and Van 1989) (Fig. 4).

Scar densities at site BP were similar to levels at site IN (Fig. 1), but leaves on plants at site BP were similar in area to leaves at sites with low scar densities, and were often larger than leaves at site IN (Fig. 2). High root and dead biomass at site BP occurred in plants that also had high above-water biomass (Fig. 3), in contrast to site IN. Feeding by *Neochetina* spp. adults was thus not coupled to plant stress. A mechanical removal event and occasional plant movement related to water flow disturbed plant patches at site BP, whereas patches were not disturbed at site IN. Disturbance could have increased the growth and compensatory response capabilities of surviving and regrowth/replacement plants at site BP (Center and Durden 1986, Center et al. 1999a). Transient stress related to weevil feeding may have occurred during Spring 2002, when plants at both IN and BP had low shoot-to-root ratios. Spring 2002 was the only time at which root and above-water biomass were not correlated. It is surprising that weevil populations were never particularly high at site BP (Fig. 5), because high adult densities might have been needed to maintain elevated scarring levels on these plants. Sampling times may have missed peaks in adult weevil abundance. In northeastern Texas, adult populations peak in late summer (Grodowitz et al. 1991). The seasonal pattern in scarring at site IN agrees with past examinations of plant damage (Center 1985) but may have been obscured by vigorous regrowth in Spring 2002 at site BP.

Variation in waterhyacinth weevil density is common (Center and Dray 1992, Center et al. 1999a) and is usually related to leaf damage (Wright and Center 1984). Adult weevil densities were positively associated with both root and dead biomass in previous regional surveys of waterhyacinth populations (Grodowitz et al. 2000). Combined egg, larval, and adult densities were not associated with scarring or biomass in the current study (Fig. 5), and densities of adults were often too low to analyze. The results demonstrate the greater accuracy of separate adult and larval densities in evaluating biomass associations. Weevil quality can also be important. Sex ratio, female reproductive capacity, and flight muscle development all show strong relationships with plant stress and phenological stage (Center and Dray 1992, Center et al. 1999a) and also vary seasonally (Grodowitz et al. 1997). Larvae are more important than adults in caus-

ing waterhyacinth leaf mortality (DeLoach and Cordo 1976, Center 1985).

Furled waterhyacinth leaves usually contained more total soluble protein than older leaves (Table 3). Total leaf nitrogen content is highest in furled waterhyacinth leaves (Center and Wright 1991). Protein or nitrogen concentrations are higher in young tissues of many plants (Awmack and Leather 2002). High nutritive quality contributes to the strong preferences of adult *Neochetina* spp. weevils for young leaves (Center and Wright 1991). Weevil feeding decreases nitrogen content in young unfurled leaves (Center and Van 1989). Protein and scar densities were negatively correlated across all sites and times (Fig. 4), although only in furled leaves, in Spring 2002, were both of the sites with high scarring (IN and BP) lower in protein than the other sites (Table 3). Protein levels were significantly lower in all leaves at site BP in Spring 2002. This result seems to contradict the conclusion that plants at site BP were more vigorous than at site IN. Waterhyacinth leaf nitrogen is often higher at sites previously disturbed by removal than at unmanaged sites (Center et al. 1999a). Nitrogen and plant vigor are also positively related in giant salvinia (Room et al. 1989). The results support the conclusion that transient stress related to weevil feeding occurred at site BP early in the growth season. Fluctuations in nitrogen or other water nutrients could also have led to reduced early-season protein content at site BP.

Peroxidase activities were highest in oldest unfurled leaves (Table 4) and are positively related to age- or stress-related leaf senescence in many land plants (Abeles et al. 1988, Bi and Felton 1995). The high peroxidase activities in Fall 2001 samples also suggest an association with senescence. Insect feeding increases peroxidase activities in tomato and other plants (Bi and Felton 1995). Peroxidases are involved in cross-linking and lignin formation (Nicholson and Hammerschmidt 1992), which can enhance leaf toughness. Feeding by weevil adults increases waterhyacinth leaf toughness, as does aging (Wright et al. 1989). In this study, peroxidase activities did not differ in young leaves between low- and high-scarring sites (Table 4), and activities and scarring were not correlated across all sites and times. Polyphenoloxidases, which are localized in phenol cells in waterhyacinth leaves (Martyn et al. 1979), may also contribute to toughness.

Canopy height and shoot density are indicators of plant biomass and asexual reproduction, respectively. Canopy height decreases over time in waterhyacinth populations that are hosting biocontrol agents (Center et al. 1999a). Augmentative releases of waterhyacinth weevils reduce shoot densities (Center et al. 1999b), although densities may increase in the short term (Grodowitz et al. 1991), especially at field sites subjected to mechanical control (Goyer and Stark 1984). No associations between canopy height or shoot density and leaf scarring were evident in this study (Fig. 6). Information about the impact of *Neochetina* spp. weevil larvae, and other biocontrol agents is needed to assess effects on plant populations.

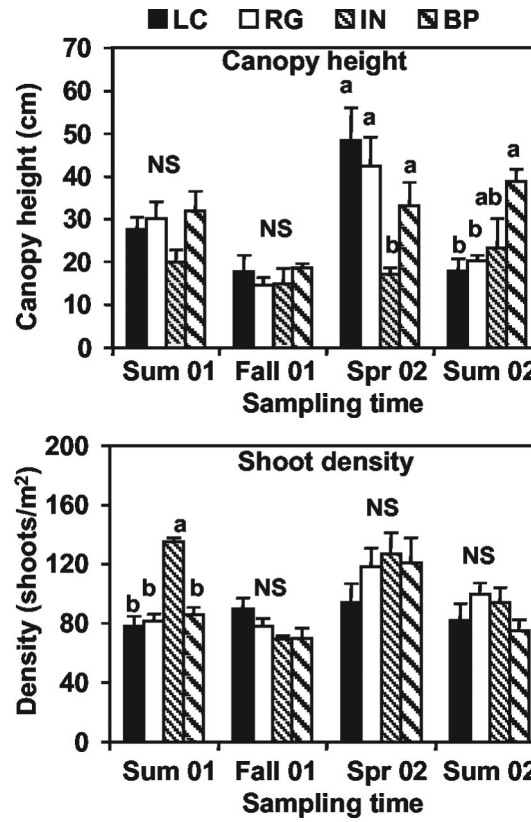

Fig. 6. Population measures of waterhyacinth plants at four sites. Sampling time abbreviations as in Fig. 2. Each bar represents the mean ± SE of four to five samples. Means marked by the same letters within each sampling time are not significantly different at $P < 0.05$. NS, no significant differences between sites.

Combined egg, larval, and adult weevil densities were negatively correlated with shoot density (P. J. M., unpublished data). Weevil dispersal into the lower Rio Grande Valley is relatively recent (Grodowitz et al. 2000). More time could be needed for population-level impacts to emerge. Leaf scarring by waterhyacinth weevils clearly affected biomass and biochemical measures in individual plants. These effects were temporally dynamic, and the status of the weevil–plant relationship varied within plants as well as between sites. Variation among sites in water flow and the use of other control methods limited the efficacy of biological control and the value of leaf scarring as an indicator of control.

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